

## Minireview

## Glycoprotein folding and the role of EDEM1, EDEM2 and EDEM3 in degradation of folding-defective glycoproteins

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**Abstract** Proteins synthesized in the endoplasmic reticulum (ER) lumen are exposed to several dedicated chaperones and folding factors that ensure efficient maturation. Nevertheless, protein folding remains error-prone and mutations in the polypeptide sequence may significantly reduce folding-efficiency. Folding-incompetent proteins carrying *N*-glycans are extracted from *futile* folding cycles in the calnexin chaperone system upon intervention of EDEM1, EDEM2 and EDEM3, three ER-stress-induced members of the glucosyl hydrolase 47 family. This review describes current knowledge about mechanisms regulating folding and disposal of glycoproteins.  
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**Keywords:** Endoplasmic reticulum; Protein folding; ER-associated degradation (ERAD); EDEM1; EDEM2; EDEM3

## 1. Introduction

Each protein is composed of a specific amino acid sequence that, to become biologically active and to be transported at the appropriate intra- or extracellular destination, must fold into a defined three-dimensional structure, usually the most thermodynamically stable. *N*-glycosylation, the co-translocational addition of pre-assembled core glycans to nascent polypeptide chains emerging in the ER lumen, facilitates the folding process by recruiting members of the calnexin (Cnx) chaperone system. This is an ensemble of sugar (*glucose*)-binding lectins, sugar (*glucose*)-modifying enzymes and dedicated oxidoreductases that retains newly synthesized proteins in a folding-friendly environment and assists rate-limiting steps of the folding process. Acquisition of the protein's native structure may fail or may progress unacceptably slowly, as in the case of mutations in the amino acid sequence. Activation of a disposal machinery that removes folding-defective and terminally misfolded products from the folding compartment and transport them across the ER membrane into the cytosol for proteasomal degradation is crucial in these cases to maintain cell and organism homeostasis [1]. The ER disposal machinery comprises sugar (*mannose*)-binding lectins and sugar (*mannose*)-modifying enzymes and takes advantage of the *N*-gly-

cans displayed on the misfolded polypeptide chains to interrupt *futile* folding attempts and to initiate the degradation program [2,3]. A detailed comprehension of the mechanisms of protein folding and protein degradation in the ER is of crucial importance because several human diseases are characterized by mutations in the polypeptide sequence that severely impairs the folding process. If the defective polypeptide chain undergoes aggregation, the disease is characterized by a gain-of-toxic function phenotype (e.g. liver disease in the  $\alpha$ 1-antitrypsin deficiency); if, on the other hand, the defective polypeptide is rapidly degraded, the disease is characterized by a loss-of-function phenotype (e.g. cystic fibrosis) [4].

## 2. Glycoprotein folding and ER quality control

### 2.1. Chaperone-assisted protein folding in the ER

The ER is the site of synthesis and folding of secretory proteins and of proteins operating at the plasma membrane or in intracellular compartments such as the ER, the endosomes, the lysosomes and the Golgi [1]. Formation of disulfide bonds and addition of pre-assembled core glycans composed of three glucose (Glc), nine mannose (Man) and two *N*-acetylglucosamine (GlcNAc) residues (Fig. 1A) are peculiar co- and post-translational modification of proteins synthesized in the ER.

The addition of core glycans (*N*-glycosylation) by the oligosaccharyl transferase (OST) occurs co-translationally as soon as a conserved Asn-Xxx-Ser/Thr consensus amino acid sequence enters the ER lumen (Fig. 2, step 1) [5]. *N*-glycosylation increases the overall solubility of as yet unstructured newly synthesized chains, recruits chaperones and folding factors dedicated to assistance of glycosylated chains, serves as a hydrophilic appendix that signals when the time allocated to complete the folding program has expired (see below). Terminal glucoses are rapidly removed by ER-localized  $\alpha$ -glucosidases to form the Glc<sub>1</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> sugar composition. In particular, the sequential removal of the two outermost glucose residues belonging to the *N*-glycan's branch A is mediated by the glucosidase I (GI) and the glucosidase II (GII) (Fig. 2, step 2) [5]. GI removes the first glucose as soon as the *N*-glycan has been transferred on the nascent polypeptide chain. Recent data have demonstrated that GII, composed by the catalytic subunit  $\alpha$  and by the mannose 6-phosphate receptor like domain  $\beta$ , can cleave the second glucose only if its  $\beta$  domain interacts with another *N*-glycan (transactivation) [6]. The trimming of the glucose in position two is therefore slower but may occur before the synthesis of the polypeptide chain is completed.

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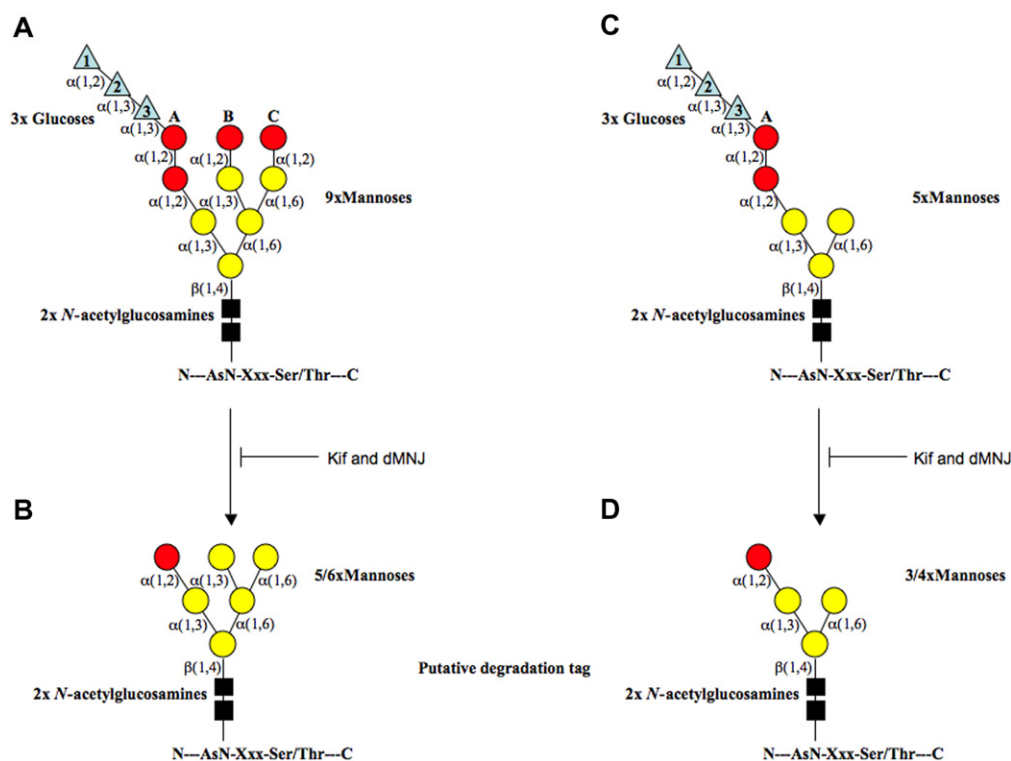


Fig. 1. N-glycan composition. (A) The precursor oligosaccharide added to the nascent polypeptide is composed of two N-acetylglucosamine (black squares), nine mannose (yellow and red circles) and three glucose residues (blue triangles). Mannose residues linked with an  $\alpha(1,2)$  bond are shown as red circles. The three N-glycan branches are labelled as A, B and C and the three glucose residues are labelled as 1, 2 and 3. Mannoses removal is necessary to generate the sweet degradation tag indicated in (B). (C) The precursor oligosaccharide added to the nascent polypeptide in the B3F7 cells is composed of only four mannose residues. (D) the putative degradation signal of misfolded glycoproteins synthesized in B3F7 cells. Kifunensine (Kif) and deoxymannojirimycin (dMNJ) inhibit mannose removal and ERAD.

The removal of the two outermost glucoses is essential for the entry of the nascent chain in the glycoprotein-dedicated Cnx/calreticulin (Crt)-ERp57 chaperone system [5] (Fig. 2, step 2).

Cnx, a membrane-bound protein, and Crt, its soluble homolog, are monomeric, calcium-binding, ER-resident chaperones that contain a sugar binding domain and a proline rich P-domain. Cnx and Crt have the identical carbohydrate specificity for mono-glucosylated trimming intermediates of polypeptide-bound oligosaccharides but associate with distinct substrate populations [3]. In addition to the glucose 3 at least three mannose residues of the branch A (Fig. 1A) critically contribute to substrate association with Cnx/Crt. The binding-affinity is low ( $\mu\text{M}$  to  $\text{mM}$ ) and the interaction is transient. Whether Cnx or Crt can interact directly with the polypeptide chain is matter of ongoing investigation [7].

The P-domain of Cnx and Crt mediates the interaction with ERp57 [8,9], a member of the protein disulfide isomerase (PDI) superfamily. Association with Cnx/Crt makes of ERp57 the glycoprotein-dedicated oxidoreductase. Disulfide bonds are important for the stability of the native protein structure, and the mispairing of cysteine residues can prevent proteins from attaining the native conformation. Oxidative folding consists in a co-translational phase during which nascent chain cysteines are oxidized to form intra-molecular disulfides and in a post-translational phase, where disulfides are rearranged into the native set. Studies of the oxidative folding of influenza virus hemagglutinin (HA) revealed that ERp57 plays a crucial role in the isomerization of non-native disulfide bonds occur-

ring during the post-translational phases of the HA folding program [10].

Thus, entry in the Cnx chaperone system protects nascent chains from aggregation and facilitates the polypeptide folding process by recruiting the dedicated oxidoreductase ERp57.

The initial stage in the folding of the glycoproteins is conserved in *S. cerevisiae* where Cne1p has been identified as the functional ortholog of Cnx [11] (a Crt ortholog has not been identified). The yeast Cne1p has a short cytosolic domain, a lectin domain that mediates association with mono-glucosylated substrates and a conserved P-domain that mediates the interaction with Mpd1p, a putative ERp57 ortholog [12]. Cne1p, as expected, works as a chaperone in the yeast ER and enhance folding efficiency of newly synthesized proteins [11]. The major difference between glycoprotein folding between *S. cerevisiae* and mammalian cells is the lack of a functional ortholog of the mammalian quality control sensor UDP-glucose:glycoprotein glucosyltransferase (UGT1, see below).

## 2.2. Cnx, Crt and ERp57 knock out mice and cell lines

To better understand the mechanism of glycoprotein folding, knock out mice that either lack Cnx [13] or Crt [14] or ERp57 [10,15] have been generated. The Cnx knock out mice show growth defects and motor disorders resulting in premature death. The Crt and ERp57 knock out mice die in uterus. Thus, even though the folding machinery appears to be highly redundant with several chaperones and folding factors displaying similar substrate specificities and/or enzymatic activities

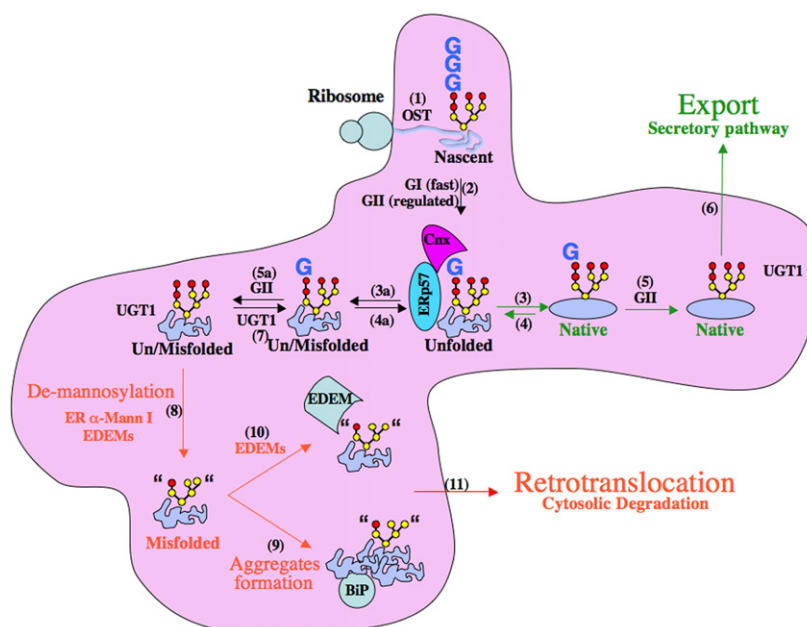


Fig. 2. Folding, quality control and ER-associated degradation. The core oligosaccharide is co-translationally added to the nascent polypeptide by the OST (1). Glucose residues are indicated as "G" and mannose residues as circles ( $\alpha$ 1,2-linked mannoses are in red). The two outermost glucoses are removed sequentially by the GI (fast) (2) and by the GII (regulated) (2). Cnx/Crt bind the mono-glucosylated *N*-glycan thus exposing the newly synthesized glycoprotein to ERp57. Native proteins: Once released by the chaperone complex, the outermost glucose is removed by the GII (5). Native proteins are released from the ER and transported at the appropriate destination (6). Folding intermediates or terminally misfolded glycoproteins: Folding intermediates are released by the Cnx/Crt-ERp57 complex (3a) and the outermost glucose is removed by the glucosidase II (5a). UGT1 re-glucosylates glycoproteins with "pseudo-native structure" (7) resulting in a second round of association with Cnx/Crt/ERp57 (4a). Terminally misfolded polypeptides become substrates of ER-resident members of the GH47 family, such as ER  $\alpha$ 1,2 mannosidase I and EDEMs (8). Misfolded proteins might be retained in the ER by the formation of size regulated aggregates containing BiP (9) or by interacting with EDEM1 that prevents aggregates formation (10). Non-native polypeptides are eventually retro-translocated into the cytosol for proteasomal degradation (11).

(e.g. ERp57 is only one of about 20 distinct ER-localized members of the PDI superfamily), individual deletions may have severe phenotypes possibly related to specific requirement of the deleted protein during embryogenesis or in the function of specific organs.

Analysis of several model glycoproteins in cell lines derived from embryos lacking individual ER-resident factors shed light on mechanisms regulating glycoprotein folding and quality control in the mammalian ER. The knock out cells showed similar viability, proliferation rate, morphology and stress levels compared to wild type cells. However, select folding substrates suffered of characteristic maturation defects upon depletion of individual chaperones. For instance, influenza virus HA proved dependent on Cnx [16,17] and the MHC class I heavy chain on Crt [18] for efficient maturation and transport at the plasma membrane. Maturation of HA [10], of class I [15] and of several proteins lacking defined secondary structures and bearing a large number of disulfide bonds also rely on ERp57 [19]. Thus, although normally exploited by a plethora of gene products synthesized in the mammalian ER, the Cnx/Crt-ERp57 system seems to be strictly required for a restricted fraction of polypeptides only.

Besides general chaperones and folding enzymes the ER also contains several substrate-dedicated chaperones (e.g. the collagen-specific Hsp47) and the LDL receptor specific RAP (thoroughly reviewed in [1]).

### 2.3. Protein quality control in the ER lumen

Immediately after substrate release from Cnx/Crt (Fig. 2, step 3), the GII removes the innermost glucose of the *N*-gly-

can's branch A (Fig. 2, step 5). This inhibits re-association of the glycoprotein with the glucose-binding lectins (Fig. 2, step 4). The structure of the *free* glycopolyptide is checked by the UGT1. UGT1 ignores native polypeptides that can leave the ER through the secretory line (Fig. 2, step 6). It also ignores terminally misfolded proteins that are deviated into the disposal machinery and prepared for retro-translocation into the cytosol and proteasome-mediated degradation (Fig. 2, step 8) [3]. UGT1 selectively re-glucosylates the mannose on branch A of polypeptides with nearly native conformation (Fig. 2, step 7) [20] thereby causing their re-association with calnexin/calreticulin-ERp57 for further folding attempts (Fig. 2, step 4a).

It is not fully understood how UGT1 can distinguish native and non-native structures, but it is reasonable to think that this depends on the presence of exposed hydrophobic residues, mobile loops, non-native determinants or the lack of compactness. Also, different models have been proposed on the requirements for *N*-glycans re-glucosylation. A first model claims that re-glucosylation only occurs if the *N*-glycan is adjacent to the structural defect in the polypeptide chain [21,22], a second model claims that *N*-glycans are re-glucosylated independent on structural-defect's proximity [23].

### 2.4. UGT1 knock out mouse and cell lines

Deletion of UGT1 is embryonic lethal in mice, with only few mice surviving until birth [24]. Generation of embryonic fibroblasts allowed, however, some studies on mechanisms regulating protein quality control in the mammalian ER. It has been reported that association with Cnx/Crt and UGT1 activity are dispensable for retention in the ER of folding-defective

glycoproteins [24]. Although UGT1 prolongs association of folding-incompetent glycoproteins with Cnx, terminally misfolded polypeptides are in fact eventually handed off from Cnx to BiP (Fig. 2, step 9), a member of the heat shock 70 protein family. UGT1 offers to folding-defective glycopolypeptides additional chances to correct their structure, but it is association with BiP of the substrates escaping the Cnx chaperone system that ensures tightness of ER quality control, independent on presence/absence of UGT1 [24].

Lack of substantial ER stress upon UGT1-deletion shows that, at least in cultured cells, most of the cellular proteins may acquire a transport-competent structure in a single binding-event with Cnx/Crt-ERp57 [24], as it is also the case for proteins synthesized in *S. pombe* where the UGT1 ortholog is present but dispensable for viability under normal conditions [25].

### 3. Degradation of folding-incompetent proteins from the ER

At the beginning of the 1990s the degradation of misfolded proteins synthesized in the ER lumen was thought to occur in the ER lumen and to be operated by ER-resident proteases [26]. It was consequently named “ER degradation”. In the mid 1990s, the concept of “ER-associated degradation” replaced the “ER degradation” model as cumulating data showed that misfolded proteins synthesized in the ER were retro-translocated into the cytosol and degraded by the 26S proteasome [27,28]. Since then, the mechanisms regulating ERAD have been thoroughly studied, in particular those regulating disposal from the ER of folding-defective proteins carrying *N*-linked oligosaccharides.

#### 3.1. Mannose trimming... the signal for destruction

Data showing that removal of  $\alpha$ 1,2-bonded mannose residues from *N*-glycans displayed on misfolded polypeptides was required for their disposal from the ER [29] were published even before data showing that the 26S proteasome was the proteolytic machinery operating degradation (reviewed in [30]). For most if not all glycosylated ERAD substrates, sugar analogs such as kifunensine or deoxymannojirimycin-derivatives

are as efficient as proteasomal drugs in inhibiting protein degradation from the ER [31]. Kifunensine and deoxymannojirimycin-derivatives are cell permeable compounds that selectively target ER and Golgi-resident members of the glycosyl hydrolase 47 (GH47) family comprising the ER  $\alpha$ 1,2 mannosidase I, the Golgi  $\alpha$ 1,2 mannosidases IA, IB and IC and the three ER-resident proteins EDEM1, EDEM2, EDEM3 (Fig. 3). Which of these is (are) involved, and how, in glycoprotein disposal from the ER is matter of intensive study.

Genetic evidence in *S. cerevisiae* shows a clear involvement of Mns1p [32] and of Htm1p [33], the ER  $\alpha$ 1,2 mannosidase I and EDEM1 orthologs, respectively. Mns1p specifically removes from *N*-glycans of folding-defective polypeptides the outermost mannose of branch B generating a strong signal for disposal. Htm1p would then act as a mannose<sub>8</sub>-binding lectin [33], even though results are controversial as other studies report that Mns1p activity upon the ERAD substrates (thus, substrate de-mannosylation) is not required for Htm1p intervention [34]. The specificity of Mns1p for mannose B (Fig. 1A) is conserved in the mammalian ortholog. The ER  $\alpha$ 1,2 mannosidase I can, however, also remove other  $\alpha$ 1,2-bonded terminal mannoses at rather unphysiological conditions such as high concentrations and prolonged incubations *in vitro* [35].

In the mammalian ER the situation is much more complex and, importantly, removal of a single mannose residue does not seem to be sufficient to elicit disposal from the ER of misfolded glycopolypeptides [2,36]. The main difference between mammalian and yeast mechanisms regulating protein biogenesis/disposal is that *S. cerevisiae* lacks a functional UGT1 homolog. Hence, substrate release from the yeast Cnx chaperone system is virtually irreversible and polypeptides can directly be deviated in the disposal machinery. On the contrary, folding-defective glycopolypeptides can be retained in the mammalian Cnx system as long as they display *N*-glycans with a re-glucosylatable mannose A (Fig. 1A). Consistently, cumulating data support a model in which extensive substrate de-mannosylation to mannose<sub>5–6</sub> structures lacking mannose A is required for protein degradation from the mammalian ER (Fig. 2, steps 8–10) [37–40]. The most compelling are probably those showing that mannose removal remains

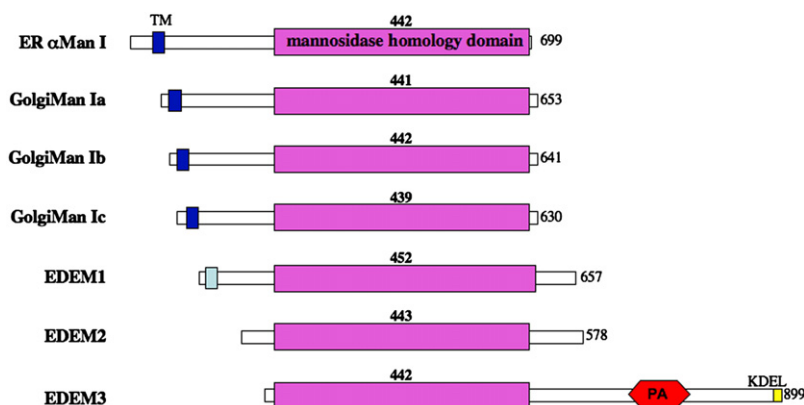


Fig. 3. Schematic representation of GH47 family members. The GH47 family comprises three subfamilies of proteins including the ER  $\alpha$ 1,2 mannosidase I, a collection of Golgi  $\alpha$ 1,2 mannosidases (GolgiManIA, IB and IC) and three EDEM proteins (EDEM1, EDEM2 and EDEM3). The conserved mannosidase homology domain is in violet. There is little or no conservation among family members beyond this domain. EDEM3 contains a protease-associated domain (PA) and the KDEL retention signal. The transmembrane regions of the ER  $\alpha$ 1,2 mannosidase I, and of GolgiManIA, IB and IC is shown in dark blue. The putative transmembrane region of EDEM1 is indicated in light blue.



required for protein degradation in mannosylphosphoryldolichol-deficient cell lines (e.g. B3F7 [41] and MadIA214 [42] (Fig. 1C)) characterized by addition of aberrant oligosaccharides on nascent chains where the only cleavable  $\alpha$ 1,2-bonded mannoses are those on branch A [43,44].

### 3.2. Members of the GH47 family involved in the extensive de-mannosylation of ERAD candidates in the mammalian ER

The GH47 family members (Golgi  $\alpha$ 1,2 mannosidases IA, IB and IC, ER  $\alpha$ 1,2 mannosidase I and EDEM1, EDEM2, EDEM3) have been implicated in disposal of misfolded glycoproteins from the ER lumen based on the genetic data in *S. cerevisiae* and/or because their specific inhibitors deoxymannojirimycin and kifunensine are potent ERAD inhibitors (see above).

Golgi members of the GH47 family catalyze mannoses removal from branches A and C and, with much lower efficiency, from branch B [45]. Their intervention in glycoprotein disposal from the ER would require traffic of ERAD candidates in the Golgi compartment (which has not been shown in mammalian cells) or residence of a sub-fraction of these enzymes in the ER. An intervention of the ER  $\alpha$ 1,2 mannosidase I is more likely, even though kinetic studies have shown that extensive *N*-glycan processing beyond removal of the mannose B requires high enzyme concentrations or prolonged incubations [35] that can only be envisioned if this mannosidase would be sequestered in highest amounts in a specialized sub-compartment of the ER.

Another model claims that members of the third subfamily of GH47 enzymes comprising EDEM1, EDEM2, and EDEM3 are directly, or indirectly involved in substrate de-mannosylation. Consistently, recent data indicate that the overexpression of EDEM1 and EDEM3 enhances mannose removal from misfolded glycoproteins (Fig. 2, step 8) [44,46].

### 3.3. The EDEM proteins

The mouse EDEM1 gene is transcribed into two mRNA species of 2.4 and 6 kb originating from different polyadenylation sites [47]. EDEM2 and EDEM3 have been identified subsequently by a data base search and their involvement in ERAD has been already assessed [46,48,49]. Based on the UniGene expression profile, the three proteins are frequently expressed in the same organs but with some difference. For example, EDEM2 is present alone in the adrenal gland and EDEM1 in the parathyroid. EDEM3 differs from EDEM1 and EDEM2 for the presence of a protease-associated domain of unknown function and for the presence of the ER retention signal KDEL (Fig. 3). It is not yet clear if the role of the EDEM proteins is redundant in the cell. Alignment of EDEM1, EDEM2, EDEM3 with the other ER- and Golgi-resident members of the GH47 family reveals conservation of about 440 residues, the so-called mannosidase homology domain amongst family members (Fig. 3). There is little or no conservation beyond this domain [48,49]. More importantly (see below) EDEM proteins conserve all catalytic residues required for glycolytic activity and structural modelling indicates no difference in their spatial location [30]. The topology of EDEM1 is controversial as it has been initially described as a type-II ER transmembrane protein in COS cells [47], with the uncleaved signal sequence as transmembrane region associating with the membrane-region of Cnx to form a functional complex [50]. However, specific algorithms (e.g. Signal P [49]) predict efficient cleavage of

the EDEM1 signal peptide, with low probability (less than 1%) to serve as a membrane-anchor. Consistently, more recently analysis showed that EDEM1 is a luminal protein in HEK293 cells [49]. These contrasting results might be explained by variations in signal peptide cleavage in different cell lines. EDEM2 and EDEM3 are soluble proteins of the ER [46,48,49].

The intraluminal level of EDEM1, EDEM2 and EDEM3 is adapted to variations of ER cargo load (e.g. upon differentiation of B cells in antibody-secreting plasma cells) or in response to accumulation of misfolded polypeptides in the ER lumen. These conditions activate a transcriptional program regulated by the ER stress-sensor Ire1 and the Ire1-dependent transcription factor Xbp1. Ire1 is a multifunctional transmembrane protein characterized by a kinase and an endoribonuclease domain in its cytoplasmic C-terminal portion. In response to ER stress, Ire1 dimerizes [51] and undergoes autophosphorylation that activates the endoribonucleolytic function. Activated Ire1 performs an unconventional splicing leading to intron removal from the mRNA coding for Xbp1, an UPR-specific  $\beta$ -ZIP transcription factor [52]. Intron removal results in a frame shift that leads to synthesis of a more stable gene product acting as a more potent transcription factor [52]. The spliced Xbp1 product binds to specific ER stress-responsive *cis*-acting elements thereby activating the transcription of select genes involved, as an example, in ERAD [53]. EDEM1, EDEM2 and EDEM3 are amongst the gene products induced upon activation of the Ire1/Xbp1 stress-response pathway [49,53].

Overexpression of recombinant EDEM1 [47,50,54], EDEM2 [48,49] and EDEM3 [46], thus conditions that mimics acute ER stress, accelerates release of terminally misfolded glycoproteins from the Cnx chaperone system [50,54] thereby accelerating their elimination from the ER lumen. On the other hand, reduction of the endogenous EDEM1 level obtained by RNA interference [54,55] or inactivation of the Ire1/Xbp1 pathway that would induce EDEM proteins expression upon ER stress decrease efficiency of glycoprotein's ERAD and eventually compromise the cell's secretory capacity [53,56].

### 3.4. How do EDEM proteins assist glycoprotein degradation from the ER?

Despite conservation of the mannosidase domain, the *S. cerevisiae* ortholog of EDEM1 is enzymatically inactive [33,57] and has been proposed to act as a lectin that binds folding-defective proteins carrying *N*-linked glycans from which the mannose B has been removed [33]. How ERAD substrates are then delivered to the site of retro-translocation into the cytosol for proteasomal degradation remains unclear. A role for the Yos9p as a bridging protein between the machinery that extracts terminally misfolded proteins from the folding environment and the machinery that retro-translocates them across the ER membrane has been suggested [34,58,59]. Yos9p binds, in fact, misfolded glycoproteins through a lectin domain similar to the mannose-binding domain of members of the mannose-6-P receptor family and has been shown to be associated to the Hrd1p retro-translocation complex [60].

Again, in mammalian cells the machinery is more complex and not completely conserved mechanistically. Firstly, a functional ortholog of Yos9p has not yet been identified. Secondly,

a possible direct role of EDEM proteins in conducting ERAD substrates to the translocation site can be hypothesized based on data showing that EDEM1 associates with derlin2 and derlin3, two putative components of a membrane channel for protein export from the mammalian ER [61]. Moreover, bacterial toxins exploit the ERAD machinery to deliver their active subunits in the host cell cytosol. EDEM1 has been shown to participate in the retro-translocation of the active subunit of the ricin toxin [62]. Thirdly, an enzymatic activity for EDEM proteins has been postulated based on data showing faster de-mannosylation of folding-defective polypeptides upon elevation of the intralumenal level of EDEM1 [44] and EDEM3 [46]. Enhanced de-mannosylation upon overexpression is abolished by a glutamic acid to glutamine mutation (E220Q in EDEM1 and E147Q [44] in EDEM3 [46]) corresponding to the E333Q mutation that inactivates the glycanase activity of the ER  $\alpha$ 1,2 mannosidase I [45]. These data would be consistent with EDEMs being enzymatically active ER proteins even though, formally, it can still not be excluded that enhanced de-mannosylation relies on recruitment of an active mannosidase upon substrates association with the EDEM proteins. So far, no data has been generated *in vitro* showing that purified EDEMs can remove mannose residues from oligosaccharides. Of interest is that the study by Olivari et al. shows that enhanced substrate de-mannosylation upon EDEM1 overexpression also occur in the B3F7 cell line, where the only removable mannose residues are those on the *N*-glycan's branch A (see above and Fig. 1C) [44].

As it has also been shown that EDEM1 inhibits aggregation of misfolded glycoproteins released from Cnx, independent of its *putative* enzymatic activity [44,63], emerging models postulate that EDEM proteins facilitate ERAD of glycoproteins by interrupting *futile* folding-attempts in the Cnx chaperone system. This results from enhanced removal of mannose residues, including the re-glucosylatable mannose *A*. The lectin chaperone activity of EDEMs also prevents entry of folding-defective proteins in off pathways characterized by formation of covalent aggregates that would delay protein disposal from the ER [64]. EDEM's association with translocon components would actually also promote substrate extrusion from the ER lumen.

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